PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Eric A. JOHNSON et al.

Application No: 07/837120 Group Art Unit: 1808

Filed: 14 February 1992 Examiner: H. Lilling

For: PROCESS FOR IN VIVO PRODUCTION OF ASTAXANTHIN AND PHAFFIA RHODOZYMA YEAST OF ENHANCED ASTAXANTHIN CONTENT

DECLARATION UNDER 37 C.F.R. 1.132

FILED

SEP - 3 1993

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Stephen Hiu, do hereby declare and state:

I hold a Ph. D. degree.

I am President of IGENE Biotechnology, Inc., assignee of the above-reference application by virtue of an Assignment recorded at Reel 4952, Frame 0724.

I reviewed the Office Action of 9 March 1993, wherein the Examiner disputes the reproducibility of the instant invention, unavailability of starting materials and enablement of the claimed invention and thus objects to the specification and rejects claims 11-13 and 28-40 under 35 U.S.C. § 112, first paragraph.

In response thereto, I reviewed the laboratory records of the instant inventors or of technicians who worked under the direction and supervision of the instant inventors to provide the following summary of data which establishes the reproducibility of the methods taught in the instant application and the sufficient enablement of the claimed invention.

To provide a proper frame of reference, it is noted that the strains set forth in the instant application are coded. Referring to instant Figure 2, the following Table provides the synonymous original strain and patent strain designations.

Original Designation	Patent Designation
Ant-1	IGI887J0
Ant-1-4	IGI887J2
104K	IGI887J3
104K2	IGI887J4
K2	IGI1287J1
K20	IGI887JI
Ant-1-460	IGI2880B60

Experiment 1

Strain Ant-1-4 (IGI887J2) was treated with nitrosoguanidine (NTG) and plated onto YM media as taught in the instant application. Approximately 600 yeast were plated per petri plate.

The plates were assessed visually for enhanced pigment level. The individual colonies were cloned and amplified. The individual colonies were grown in YM broth as described in the instant application.

Then, the level of astaxanthin was determined for the individual isolates.

Amount of Astaxanthin* per Gram Dry Yeast	Number of Strains
700-800 ppm	2
800-900 ppm	3
900-1000 ppm	2

Experiment 2

Strain Ant-1-4 was treated as in Experiment 1. The following stains within the scope of the claimed invention were obtained:

Amount of Astaxanthin* per Gram Dry Yeast	Number of Strains
600-700 ppm	1
700-800 ppm	2
800-900 ppm	9
900-1000 ppm	5
1000-1200 ppm	1
> 1200 ppm	1

* µg/g dry yeast = 1 part per million (ppm)

Experiment 3

Strain Ant-1-460 was treated as in Experiment 1 and assessed for astaxanthin content with the following results:

Amount of Astaxanthin per Gram Dry Yeast	Number of Strains
900-1000 ppm	1
1000-1100 ppm	5
1100-1200 ppm	3
1200-1300 ppm	1
1300-1400 ppm	1

Experiment 4

Strain Ant-1-460 was treated with NTG as in Experiment 1 with the following results:

Amount of Astaxanthin per Gram Dry Yeast	Number of Strains
< 1000 ppm	4
1000-1100 ppm	3
1100-1200 ppm	10
1200-1300 ppm	6
1300-1400 ppm	5
1400-1500 ppm	14
1500-1600 ppm	8
1600-1700 ppm	5
1700-1800 ppm	1

1800-1900 ppm	4
1900-2000 ppm	1
> 2000 ppm	4

Experiment 5

A highly pigmented strain obtained from Ant-1-4 was irradiated with UV light as in Example 1 of the instant invention and plated on YM plates. Colonies with enhanced pigmentation were selected and amplified in YM medium.

Amount of Astaxanthin per Gram Dry Yeast	Number of Strains
700-800 ppm	3
800-900 ppm	1
900-1000 ppm	6
1000-1100 ppm	2
1220-1300 ppm	1
1300-1400 ppm	4
1400-1500 ppm	1
1500-1600 ppm	1
1600-1700 ppm	2
> 1700 ppm	1

Experiment 6

Wild-type Phaffia ATCC 24202 were selected on YM agar containing 8 μg/ml tunicamycin as taught in the instant application. Of those colonies that survived the selection, one produced 611 ppm astaxanthin in YM broth compared to 269 ppm of the parent wild-type strain.

Experiment 7

Wild-type Phaffia ATCC 24202, grown on YM media, were exposed to 5 mM mevalonic acid lactone as taught in the instant application. The amount of mevalonic acid lactone was increased to 25 mM mevalonic acid lactone. The parent strain produced 227 ppm astaxanthin. Three strains which were selected from the 25 mM plates were obtained and found to produce more astaxanthin than the parent strain. For example, one strain mev6 produced 627 ppm astaxanthin.

Clearly, the instant invention teaches a reproducible process for obtaining yeast within the scope of the instant application. In each experiment, yeast within the scope of the claimed invention were obtained.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

9/20/93
Date

Stephen Hiu

American Type Culture Collection

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

IGENE Biotechnology, Inc.
Attention: Dr. Stephen F. Hiu
9110 Red Branch Road
Columbia, MD 21045

Deposited on Behalf of: IGENE Biotechnology, Inc.

Identification Reference by Depositor:	**ATCC Designation**
Phaffia rhodozyma, Ant-1 (IGI887J0)	66270
Phaffia rhodozyma, Ant-1-4 (IGI887J2)	66272

The deposits were accompanied by: __ a scientific description X a proposed taxonomic description indicated above.

The deposits were received June 13, 1989 by this International Depository Authority and have been accepted. A request to convert the deposits to deposits under the Budapest Treaty was received on September 30, 1993.

AT YOUR REQUEST:

X We will inform you of requests for the strains for 30 years from 1993.
X The strains are available to the scientific public upon request as of June 13, 1989.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from 1993, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested October 5, 1993. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Bobbie A. Mendez **Date:** October 6, 1993

Bobbie A. Brandon, Head, ATCC Patent Depository

✓cc: Dr. Dean Nakamura